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Temporal Development and Prognostic Value of Antibody Response to the Major Neutralizing Epitopes of gp120 During HIV-1 Infection

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Our objective was to analyse the humoral response to the major neutralizing epitopes of gp120. The kinetics of the appearance of antibodies directed to the V3 region (V3 Abs) and antibodies directed to the CD4 binding site (CD4BS Abs) were compared in sequential sera from 20 seroconverters. V3 Abs were titrated using 2 different indirect EIAs with synthetic oligopeptides coated on the solid phase. The sequences of the oligopeptides used were those of the MN isolate or a mixture of the consensus sequences of the 5 major HIV-1 subtypes (A–E). CD4BS Abs titers were determined using an EIA in which serum antibodies compete with a labeled human monoclonal antibody, F105, whose corresponding epitope overlaps the conformation-dependent CD4BS, for binding to purified recombinant gp120 coated on a solid phase. The prognostic value of both antibodies was analyzed in a longitudinal study of 60 HIV-1 infected patients (17 nonprogressors and 43 progressors). Eighty-five percent and 70% of HIV seroconverters were positive for V3 Abs and CD4BS Abs, respectively, during the observation period. V3 Abs were detected first in the majority of the patients (mean delay of appearance, 1.22 ± 0.96 months vs. 4.81 ± 2.05 months for CD4BS Abs). Both categories of antibodies appeared simultaneously in 4 patients (20%). No prognostic value could be attributed to these antibodies. Our data confirm that V3 Abs and CD4BS Abs appear with some delay after primary infection, suggesting that they do not play a large or early role in the rapid clearance of viremia in primary HIV-1 infection. These antibodies were not associated with progression to symptomatic infection and are thus of no value for surveillance in HIV-1 infected patients. ***J. Med. Virol. 52:309–315, 1997.***

KEY WORDS: humoral immunity; V3; CD4 binding cite

INTRODUCTION

During human immunodeficiency virus type 1 (HIV-1) primary infection, there is a short period of high-titer viremia [Daar et al., 1991]. The virus load then decreases very rapidly due to a vigorous immune response [Koup et al., 1994]. Investigation of serological events after seroconversion may help dissect the components of the humoral response associated with or following viral clearance.

The HIV-1 surface glycoprotein gp120 is the principal target for the neutralizing response during infection, and consequently efforts to develop a protective vaccine have centered on this protein. Neutralizing anti-gp120 antibodies in HIV-1 infected individuals are targeted to 2 major epitopes [Chamat et al., 1992; Steimer et al., 1991], although a third cluster of epitopes that mediate virus neutralization has been identified more recently [Gorny et al., 1994; Warrier et al., 1994; Wu et al., 1995]. The principal neutralizing determinant (PND) is located in the central area of the third hypervariable domain of gp120 (V3) and is part of a disulfide bridged loop. This epitope is continuous [Javaherian et al., 1989; Rucshe et al., 1988] and most antibodies directed to this region neutralize only a limited number of HIV-1 strains due to its variability [Javaherian et al., 1990; Nara et al., 1988]. A second group of neutralizing antibodies inhibit gp120 binding to the CD4 molecule and bind to a discontinuous or confor-

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mational epitope [Haigwood et al., 1992; Moore and Ho, 1993]. These antibodies neutralize a wide range of virus isolates [Steimer et al., 1991] due to the conserved nature of this epitope. The amino acids important for the binding of these conformation-dependent neutralizing antibodies are located in 7 discontinuous conserved regions of gp120 [Ho et al., 1991; Kang et al., 1991; McKeating et al., 1992; Thali et al., 1991; Thali et al., 1992]

The antibody response to the V3 epitope(s) has been studied by simple ELISAs with synthetic peptides as antigen on the solid phase [Warraen et al., 1992]. Due to the conformation-dependent nature of the CD4 binding site (CD4BS) there have been few extensive studies of the specific response to this epitope [Back et al., 1990; Cavacini et al., 1993]. Development of the anti-gp120 antibody response has been studied in depth in sequential samples from only 3 patients presenting initially with symptoms of the primary HIV-1 syndrome [Moore et al., 1994]. We developed recently a simple immunoassay able to detect specifically and quantify antibodies to the CD4BS [Turbica et al., 1995]. The assay is a competitive ELISA in which serum antibodies compete with a labeled human monoclonal antibody, F105, whose corresponding epitope overlaps the conformation-dependent CD4BS, for binding to purified recombinant gp120 coated on a solid phase [Posner et al., 1991; Posner et al., 1993]. The assay is easy to undertake, allowing the testing of a large number of serum samples. Therefore we analyzed the temporal development of the antibody response to gp120 by comparing the kinetics of appearance of both CD4BS antibodies and V3 antibodies in sequential sera from 20 seroconverters. The prognostic value of each category of antibodies was also studied in 60 HIV-1 infected patients with long term follow-up.

MATERIAL AND METHODS

Antibodies to the CD4 Binding Site

The presence and titers of antibodies to the CD4 binding site (CD4BS Abs) were determined using enzyme immunoassay (EIA) developed recently [Turbica et al., 1995]. Briefly, the solid phase (Luxlon plates, CML, Nemours, France) was coated in sodium carbonate buffer (50 mM, pH 9.6; 100 μl per well) with 0.5 μg/ml recombinant gp120 produced from Chinese hamster ovary (CHO) cells. Nonspecific binding sites were saturated for 2 h with 200 μl of a 3% solution of bovine serum albumin (BSA) in phosphate buffered saline (PBS) at room temperature. Plates were then washed 3 times with PBS buffer containing 0.5% Tween 20 (PBS-T). Sera were diluted in PBS supplemented with 0.05% Tween 20 and 1% BSA (PBS-T-BSA). Twenty microliters of diluted sera were added per well with 100 μl of a biotinylated-F105 solution diluted in PBS-T-BSA (0.005 μg/well) and the plates incubated for 1 h at room temperature. The plates were washed 3 times and incubated for 30 min at room temperature with 100 μl of a 1:5000 solution of streptavidin-peroxidase (in PBS-T-BSA). After 3 washings, the color reaction was developed at room temperature using the mixture H_2O_2/*o*-phenylenediamine (H_2O_2-OPD) as substrate. Color development was stopped after 20 min with 100 μl of 2 N H_2SO_4. Absorbance (OD) at 490 nm was measured.

The cut-off of the assay was determined for each plate using the mean OD of 4 wells corresponding to the negative control minus 0.666 [Turbica et al., 1995]. CD4BS Abs were titrated by testing serial dilutions (1:10, 1:50, 1:250, 1:1250) of the sera. The endpoint titer was given by the dilution-factor at which the absorbance was equal to the cut-off.

Antibodies to V3

Antibodies to V3 (V3 Abs) were titrated using 2 different indirect EIAs. One used a V3 synthetic oligopeptide corresponding to the sequence of the MN isolate. This isolate-specific peptide was chosen due to the high prevalence of HIV-1 MN related isolates in European patients [Zwart et al., 1991; Zwart et al., 1993]. The second V3 EIA (V3A–E EIA) used a mixture of V3 synthetic oligopeptides representing the consensus sequences of the 5 major HIV-1 subtypes A–E [Myers et al., 1992]. This assay was carried out in addition to the V3MN EIA because EIAs using V3 consensus sequences appear to be able efficiently to detect V3 antibodies in HIV-1 infected individuals of all geographical origins [Baillou et al., 1993]. The sequences of the peptides are the following:

MN	YNKRKRIHIGPGRAFYTTKNIIGTIRQAHC
A	NNTRKSVHIGPGQAFYATGDIIGDIRQAHC
B	NNTRKSIHIGPGRAFYTTGEIIGDIRQAHC
C	NNTRKSIRIGPGQTFYATGDIIGDIRQAHC
D	NNTRQRTHIGPGQALYTT.RIIGDIRQAHC
E	NNTRTSITIGPGQVFYRTGDIIGDIRQAHC

The peptides were made by a solid-phase procedure using an automated peptide synthesizer (431 A; Applied Biosystems, Foster City, CA), with Fmoc(9-fluorenylmethoxycarbonyl)-protected amino acids and hydroxymethylphenoacetic resin. After synthesis, the resin support and the side chain-protecting groups were removed with trifluoroacetic acid, using distilled water, phenol, dithioethane, and thioanisol as scavengers. After cleavage, the peptides were purified by reversed-phase chromatography on C_8 columns (Aquapore octyl, 20 μm, 100 × 10 mm; Applied Biosystems). The purity of preparations was confirmed by both the presence of a single sharp peak in HPLC and amino acid analysis. The composition of each peptide was in agreement with expectations.

Both V3 Abs assays were carried out using the same protocol except for the coating. For the V3MN EIA, the V3MN peptide was coated at 0.5 μg/ml on wells of polyvinyl microtiter plates (Falcon Microtest III, Los Angeles, CA). The peptide was diluted in sodium carbonate buffer (50mM, pH 9.6; 100μl per well) and incubated overnight at 37°C. For the V3A–E EIA, the 5 peptides were mixed at a final concentration of 0.5 μg/ml each

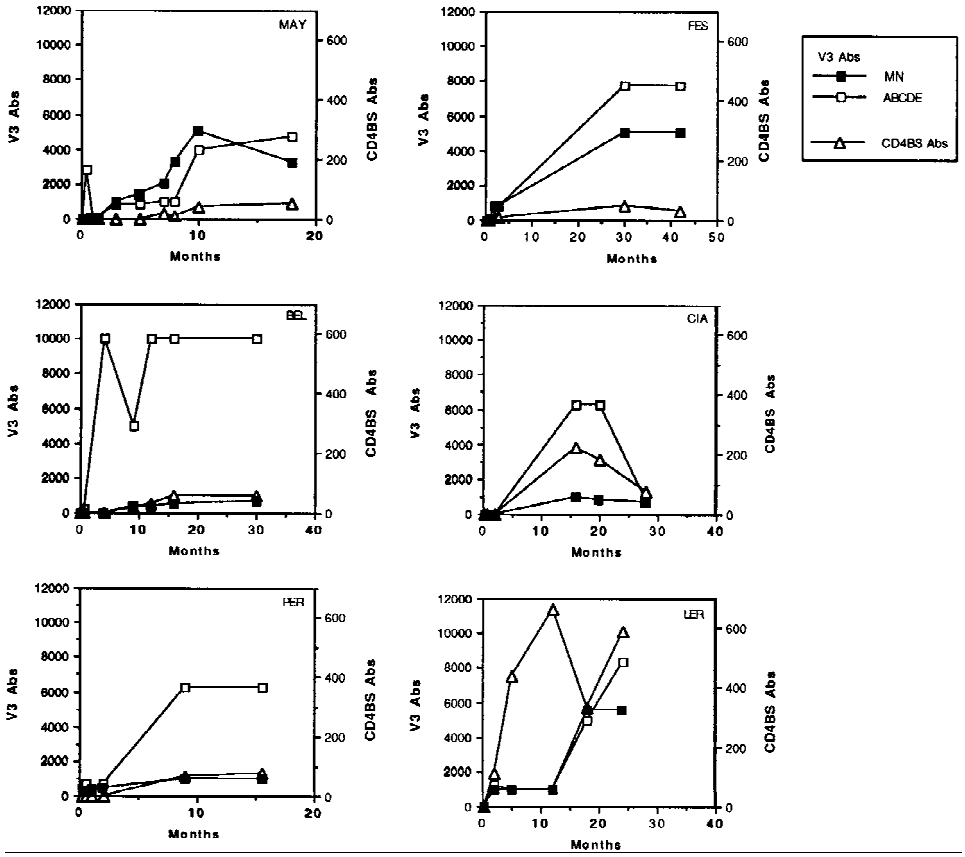

Fig. 1. Representative individual patterns of antibody response to V3 and CD4BS during seroconversion. Titers of V3 Abs are reported on the left ordinate and titers of CD4BS Abs on the right ordinate. Time 0 corresponds to the first available sample. Patient BEL was infected by a subtype C variant.

and used for coating as described above. After complete drying, plates were saturated with PBS containing 2% of newborn calf serum (NBCS) for 45 min at 37°C, and then washed 3 times with PBS-T. Sera were serially diluted in 5 × NaCl PBS buffer (0.02 M NaH2PO4, 0.02 M Na2HPO4, 0.75 M NaCl, pH 7.4) supplemented with 0.5% Tween 20 and 10% NBCS (PBS-T-NBCS). One hundred microliters of diluted serum was incubated for 30 min at room temperature and after 5 washings in PBS-T, 100 μl of peroxidase-conjugated goat F $(ab')_2$ anti-human Ig (Tago, Burlingame, CA) diluted 1:10000 in PBS-T NBCS was added and the plates incubated for 30 min at room temperature. After 3 washings, the color was developed and measured as described for the CD4BS Abs assay.

The cut-off of the V3 assays was determined by testing 190 randomly selected sera from HIV seronegative individuals at 1:10 dilution. The mean absorbance values of these negative sera were 0.0064 ± 0.0098 for the V3A–E assay and 0.008 ± 0.017 for the V3MN assay. Ten negative sera were pooled and this pool was used as a negative control for each run. The cut-off was determined for each plate as the mean OD of 4 negative control wells plus 3 standard deviations (i.e., +0.03 and +0.05 for V3A–E and V3MN assays, respectively). V3 Abs were titrated by testing serial dilutions ($1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$) of the sera. The endpoint titer was given by the dilution-factor at which the absorbance was equal to the cut-off.

Serum samples

Seroconverters. One hundred and twenty-two sequential serum samples were collected from 20 HIV-1 positive seroconverters between March 1989 and January 1995 (Bichat Hospital, Paris). For every patient, the first sample showed a typical profile of seroconversion, i.e., presence of either antibodies to p24 or antibodies to gp160 or both, and subsequent sera showed an increase in reactivities, confirming seroconversion. The follow-up of the patients allowed the testing of samples collected at short intervals, in some cases only two weeks apart.

Longitudinal study in progressors and nonprogressors. A longitudinal study was conducted with 180 sequential serum samples collected from 60 patients (Bretonneau Hospital, Tours), and the first sample was collected at CDC stage II or III (1987 classification). Three samples were tested for every patient, the first sample at entry (early sample), the second sample at approximately the mid follow-up (intermediate sample), and the last at the last visit (late sample). Seventeen patients remained at CDC stage II

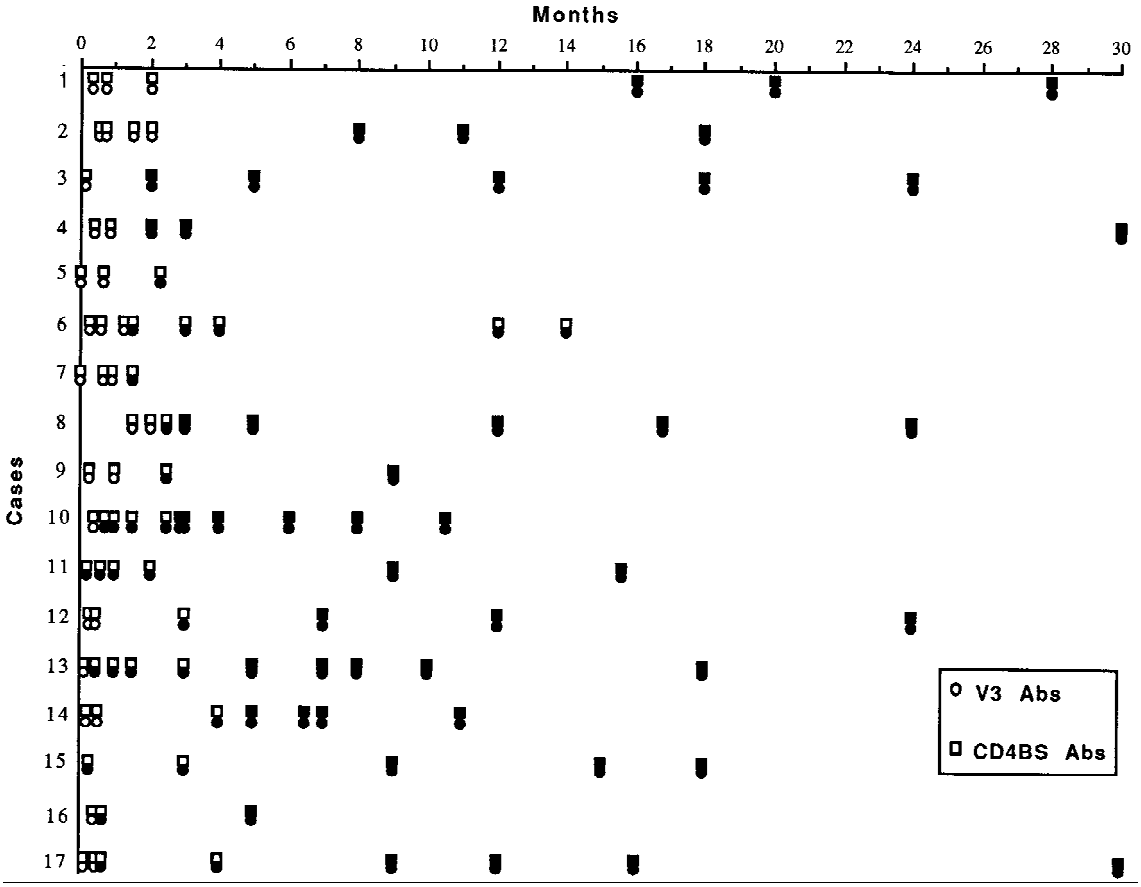

Fig. 2. Qualitative antibody response to V3 and CD4BS during seroconversion. Open symbols correspond to negative samples and dark symbols to positive samples. Time 0 corresponds to the first available sample. Cases 1–4: both categories of antibodies appeared simultaneously. Cases 5–7: no CD4BS Abs appeared during the observation period. Cases 8–17: V3 Abs were the first detected. The 3 patients who did not develop either CD4BS Abs or V3 Abs are not included in this figure.

or III (nonprogressors) and 43 progressed to CDC stage IV (progressors). The mean period of follow-up was 5.6 ± 2.3 years and 4.7 ± 1.9 years for nonprogressors and progressors, respectively.

RESULTS

Temporal Development of V3 Abs and CD4BS Abs in Seroconverters

Twenty seroconverters were tested for the presence of both categories of antibodies between 0.5 and 30 months following the collection of the first sample. Representative individual patterns of antibody response to V3 and CD4BS are shown in Figure 1 and a summary is presented in Figure 2. In most cases, the kinetics of appearance of antibodies to V3MN and V3A–E were similar. A clear preferential response to V3A–E compared to V3MN was observed for 2 cases: one in a Congolese patient, probably exposed to an HIV-1 variant distantly related to the MN prototype, and one in a Moroccan patient infected by a variant identified as belonging to the C clade [Barin et al., 1996]. Seventeen (85%) and 14 (70%) patients were positive for V3 Abs and CD4BS Abs, respectively, during the observation period. However, 3 patients in whom neither V3 Abs nor CD4BS Abs was detected were followed for only short periods, less than 2.5 months. Both categories of antibodies appeared simultaneously in 4 patients (Fig. 2). V3 Abs were detected first in 10 patients. The mean delay before V3 Abs appearance was 1.22 ± 0.96 vs. 4.81 ± 2.05 months for CD4BS Abs (difference statistically significant, $P < 10^{-4}$; Student's t-test). Three patients had no CD4BS Abs during the observation period (1.5 months, 2.3 months, and 24 months), but V3 Abs appeared early (respectively 1.5, 2.3, and 1.5 months after the first sample).

Longitudinal Study of V3 Abs and CD4 Abs in Progressors and Non-progressors

There was no significant difference in the V3 Abs titers or in CD4BS Abs titers between progressors and nonprogressors at entry (Fig. 3). Both median and mean values of antibody titers were similar in the 2 groups. To analyze the potential prognostic value of these antibodies further, the rate of change (slope) of the different antibody titers in the 3 sequential serum samples were calculated for every patient. Again, no statistically significant difference was observed between the groups, indicating the poor prognostic value

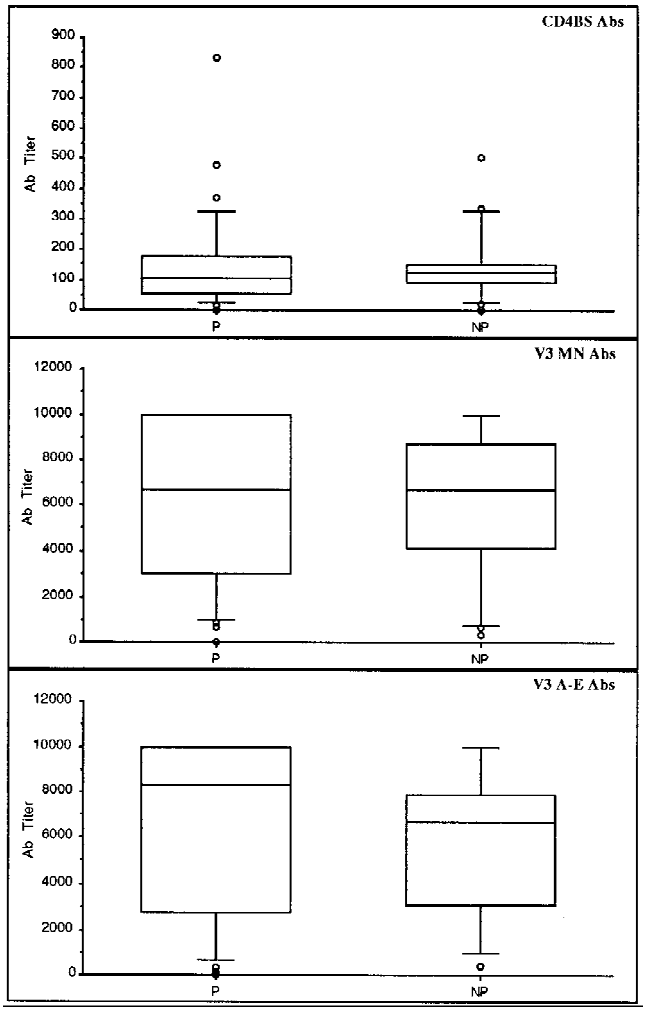

Fig. 3. Distribution of V3 Abs and CD4BS Abs titers in symptomless HIV-1 Ab positive patients at entry according to progression (P) or nonprogression (NP) to CDC stage IV. Box plots indicate the distribution and the median. The 5 horizontal lines in the boxes show the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles. Values above and below the 10^{th} and 90^{th} percentiles are represented as open circles.

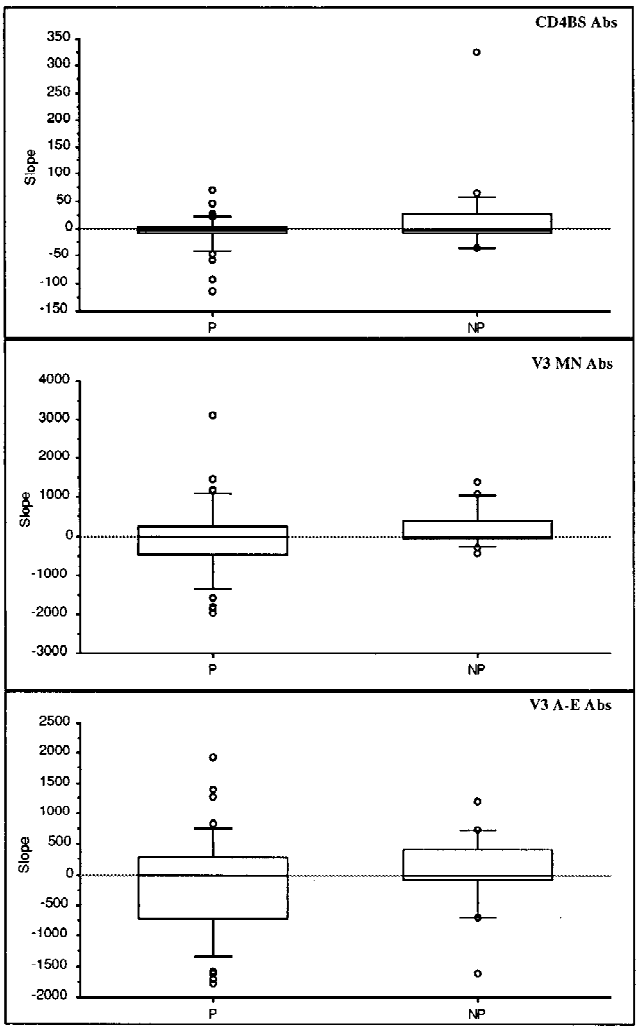

Fig. 4. Distribution of the slopes of V3 Abs and CD4BS Abs titers in followed-up patients according to progression (P) or nonprogression (NP). Box plots indicate the distribution and the median. The 5 horizontal lines in the boxes show the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentiles. Values above and below the 10^{th} and 90^{th} percentiles are represented as open circles.

of both V3 Abs and CD4BS Abs. The median value of the slopes was near 0 for every category of antibody in every group of patients, showing the stability of these antibody titers independent of the clinical progression (Fig. 4).

DISCUSSION

The study of 20 cases of HIV-1 seroconversion permitted the analysis of the temporal development of the early antibody response to the major neutralizing epitopes of HIV-1 gp120. In a recent documented study of 3 cases, Moore et al. [1994] found that CD4BS Abs were the earliest detectable anti-gp120 antibodies. In contrast, we found that V3 Abs were detected before CD4BS Abs for the majority of the patients, in accordance with other studies [Bolognesi, 1995]. Indeed, it has been considered that V3 Abs appear early and account for the homologous neutralization activity, whereas CD4BS Abs, appearing later, account for the broadening of neutralization that occurs over time [Bolognesi, 1995; Zwart et al., 1994]. These apparent discrepancies could be due to methodological differences in the assays. Moore et al. [1994] have suggested that CD4BS Abs could show some degree of strain specificity. However, for practical reasons it is difficult to evaluate for every patient CD4BS antibody binding to gp120 from the infecting virus.

The data confirm that although both V3 Abs and CD4BS Abs are detectable relatively early during seroconversion, they appear with some delay after primary infection. Therefore, unless these antibodies are effective for HIV neutralization at a concentration undetectable with such binding assays, they presumably do not contribute significantly to the rapid clearance of HIV-1 viremia regularly observed during primary infection. This is in agreement with several observations

showing that neutralizing antibodies, even to autologous strains, are also detectable relatively late compared to suppression of viremia [Ariyoshi et al., 1992; Moore et al., 1994]. These data are consistent with the cellular immune response via cytotoxic T lymphocytes being largely responsible for early control of HIV-1 primary infection [Koup et al., 1994].

Due to their major neutralizing activity in vitro, the in vivo potential role of V3 Abs has been studied extensively, but the results are divergent [Chamat et al., 1992; Javaherian et al., 1998; Warraen et al., 1992]. In contrast, very few studies have examined the potential association of CD4BS Abs with clinical outcome [Back et al., 1990; Cavacini et al., 1993; Moore and Ho, 1993]. We found no relationship between the intensity of antibody binding to either V3 sequences or the CD4BS of gp120 and disease progression. Thus no major role in controling HIV-1 associated immunodeficiency can be attributed to these antibodies, at least in established infections. However, this does not preclude the possibility that CD4BS Abs is inefficient in preventing primary infection. Our study also indicates clearly that neither V3 Abs levels nor CD4BS Abs levels can be used as prognostic markers of clinical progression.

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